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Antibiotics susceptibility patterns of bacteria associated with diabetic wound infections in selected hospitals in Awka, Anambra state, Nigeria

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Abstract

This research investigated the bacteria associated with diabetic wound infections in selected hospitals in Awka, Nigeria and their antibiotics susceptibility patterns. 115(98.3%) isolates were characterized and identified from 117(100%) study participants specimen using standard microbiological methods. The results showed that 78(67.8%) were Gram-negative and 37(32.2%) were Gram-positive. *Staphylococcus aureus* 29(25.2%) was the predominant isolate followed by *Pseudomonas aeruginosa*24(20.8%), *Escherichia coli* 21(18.3%), *Klebsiella pneumonia* 13(11.3%), *Serratia marcescens* 8(7.0%), *Proteus vulgaris* 5(4.3%), *Enterococcus faecalis* 4(3.5%), Enterobacter specie 4(3.5%), *Bacteroide fragilis* 3(2.6%), *Staphylococcus epidermidis* 3(2.6%) and *Streptococcus pyogenes* 1(0.9%). Antibiotics susceptibility testing revealed that *Pseudomonas aeruginosa*was most sensitive to Colistin (100%), followed by Gentamicin (87.5%) but resistant to Vancomycin (100%) and Erythromycin (87.5%), *Staphylococcus aureus* was most sensitive to Cefuroxime (100%), followed by Piperacillin-tazobactam (100%) but resistant to Meropenem (100%) and Ceftazidime (100%), *Escherichia coli* was most sensitive to Imipenem (90.5%) followed by Meropenem (81.0%) but resistant to Cefoxitin (100%) and Erythromycin (100%). Multidrug-resistance (MDR) profiles of the organisms showed that of the 115 bacterial isolates, 99 (86.1%) were MDR, that is resistant to more than two agents of antibiotic classes, whereas 16(13.9%) were non-MDR. Continuous surveillance to monitor etiology and antibiotics susceptibility patterns both in the community and hospital settings to guide the empirical use of antibiotics should be carried out. Increasing awareness among the population to the hazards of inappropriate antibiotics use through public health education campaigns should also be undertaken.

Keywords: Bacteria; Diabetic wound infection; Antibiotics susceptibility Testing; Gram-negative; Gram-positive; Multi-drug resistant.

1. Introduction

Wounds can result when the operative barrier of the skin is breached by traumatic or surgical invasion of the skin and adjacent tissue (1). The open area is extremely susceptible to microbial invasion and once a wound has become infected, pus forms on the injured area resulting in wound abscess. Diabetes is one of the oldest and major chronic non-communicable endocrine disorders, which may result in severe health consequences due to damage to various end organs (2). It is a lifestyle related disease which is characterized by chronic hyperglycemia.

Diabetes is of major global public health concern, afflicting a large number of people of all socio-economic status (3). The number of people living with diabetes in the world in 2017 was 435 million (4). It is estimated that approximately 15–25% of diabetic patients develop diabetic foot ulcers during the course of the disease (5). This diabetic foot ulcer incidence is the leading cause of total amputations recorded (35.4%) in Nigeria in 2019 (6).

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Diabetes mellitus is broadly categorized as; types 1, 2 and gestational. Type 1 occurs most frequently in children, Type 2 most frequent among adults accounting for 90-95% of all diabetic cases and gestational diabetes occurs during pregnancy (7). One of the serious consequences of diabetes is diabetic wound infections (DWIs) like, diabetic foot infections (DFIs), infected diabetic foot ulcer (IDFU) and its complications such as osteomyelitis (local spread of infection to muscle and bone) (8). These further may lead to repeated hospitalization, treatment failure and increased health-care expenses.

A diabetic foot infection can appear as the result of an otherwise small injury (9). These small scrapes, scuffs, and blisters, which most people experience without issue, can lead to diabetic foot ulcers. Diabetic ulcers are not infections, but they often lead to infections. These ulcers often exist without notice and can develop into serious wounds over time (9). Once the diabetic ulcer forms, pathogens can enter the blood stream and create an infection which can progress to systemic infection, septicemia, amputation or even death.

Diabetic foot infections (DFIs) are typically colonized by bacteria similar to those found in the surrounding skin and become more complex in microbial diversity over time and with progression of the ulcer (10). A previous study reported that at least 20% of the DFIs are managed by lower limb amputation (11). DFIs are common, especially in men and individuals older than 60 years (12). The development of DFI is predisposed by multiple factors such as peripheral vascular disease (PVD), peripheral neuropathy, trauma, diabetic foot ulcer (DFU), radioactivity exposure, undernourishment and impaired host immunity. However, the optimal management of DFIs and DFUs through a multi-disciplinary approach favors the better outcome in terms of reduced morbidity, mortality and health-care costs (13).

The microbiology of DFIs is often polymicrobial comprising both Gram-positive and Gram-negative aerobic bacteria and anaerobes. Studies have shown diversity in pathogens and their susceptibility patterns. *Staphylococcus aureus* was reported as a predominant pathogen associated with DFIs (3). In contrast, the predominance of Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* was observed in some studies (14, 15). Other common Gram-negative rods isolated from DFIs are *Proteus spp*. and *Acinetobacter baumannii* (16). The type of infecting microorganisms and their antibiotics susceptibility patterns differs from country to country and from one region to another within the country, economy, environment, lifestyle, and awareness (17).

Globally, multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producers and carbapenem-resistant Enterobacteriaceae (CRE) have dramatically increased in the past two decades (16). These pose a serious challenge for physicians to treat DWIs and often led to treatment failure and increased mortality. Clinicians mostly have to use initial antibiotics empirically before the result of microbial culture is available (16). False diagnosis of Diabetic wound infections (DWIs) leads to unnecessary overuse or misuse of antibiotics. Indiscriminate use of antibiotics is a major factor driving antibiotic resistance. Therefore, it is necessary to routinely assess microbes and their antibiotic resistance patterns (18).

The precise knowledge among clinicians about the pathogens and their antibiotics susceptibility patterns in a particular locality and judicious use of antibiotics is imperative for better management of DWIs and to reduce the development of antimicrobial resistance and healthcare expenses. Early diagnosis of lesions and prompt initiation of appropriate antimicrobial therapy are essential for controlling the infections and preventing complications and improving the quality of life (16).

2. Materials and methods

2.1. Materials for the study

All materials, reagents and media used for this analysis were of analytical grade and were obtained from the General Laboratory, Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria.

2.2. Study area

The prospective, observational study of patients with diabetic wound infections was carried out at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Amaku, Awka, St. Charles Medicals and Diagnostics (SCMD) Awka and Graceland Specialist Hospital and Maternity (GSHM) Awka. The hospitals are situated at the center of the town and often have a high influx of patients. The study started December, 2022 and lasted until October, 2024.

Source: Google map

Figure 1 Map view of Amaku Teaching Hospital, Awka

Source: Google map

Figure 2 Map View of Graceland Specialist Hospital and Maternity

Source: Google map

Figure 3 Map View of St. Charles Medic Diagnostics Awka.

2.3. Samples used for the study

The samples for the study were diabetic wound samples collected from 117 diabetic patients' legs such as the heels and tips of hammer toes, and arm. The clinical signs observed on the wounds were fluid discharge, foul smell, redness and swelling.

2.4. Sample collection

The wound site and size were examined with the patient laying supine on an examination table. Superficial dead tissue was removed with sterile scissors and a scalpel blade. After local debridement of devitalised tissue, the wound was cleaned with sterile saline. Samples were taken from the deepest part of the wounds using two sterile swabs. The samples were taken using a firm circular motion with the swab. One swab was used for Gram staining and the other was used for culture. In this study, ulcers were classified according to the Wagner Diabetic Foot Ulcer Classification System (19). The classification was Grade 0-Pre-ulcerative, with no open lesion or cellulitis, Grade1-Superficial ulcer, Grade2-Deep ulcer up to tendons and joint tissue, Grade 3-Deep ulcer with abscess, osteomyelitis, and joint sepsis, Grade 4-Localized gangrene of forefoot or heel, and Grade 5-Gangrene of entire foot/global gangrene. The samples were transferred to the General Laboratory, Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University in Amies transport media, and processed immediately within an hour of collection for analysis.

2.5. Kidney disease test

The method described by Cheesbrough (22) was used. A considerable volume of urine sample was collected from study participants in a clean, dry container that would allow complete immersion of all the fields on the test strip. The test strip was dipped into the urine up to the test area, ensuring that all reagent pads were fully immersed for two seconds. The edge of the strip was drawn along the brim of the vessel to remove excess urine; ensuring that the test areas did not touch the brim of the vessel. The strip was turned on its side and tapped once on a piece of absorbent material to remove any remaining urine that may cause the interaction of chemicals between adjacent reagent pads. The colour of the reagent pads was compared exactly after 60 seconds with the colour chart on the vial label under good light. The protein, specific gravity and Creatinine results as indicated on the vial label was recorded.

2.6. Skin texture examination

The study participants skin was closely observed physically and recorded as either dry, moist or cracked.

2.7. Hypertension test

The method described by Cheesbrough (22) was used with the assistance of nurses. The patients were asked to loosen any tight clothing and remove long-sleeved garments so that it is possible to access the upper arm ensuring not to use an arm that had a medical problem. The cuff was placed around the upper arm and secured. The cuff tubing was connected to the Sphygmomanometer tubing and secured. The patient's arm was rested on a surface that was level with their arm. The stethoscope was placed over the brachial artery (in the bend of the elbow) and listened to for the pulse. The cuff was pumped up slowly and listened for when the pulse disappeared which was an indication to stop inflating the cuff. The cuff was very slowly deflated whilst watching the mercury level in the Sphygmomanometer. The sphygmomanometer reading when the pulse reappeared was noted and recorded as the systolic pressure. The cuff was further delated until the pulse disappeared and recorded as the diastolic pressure. Systolic and diastolic readings of 120-129mm Hg and 80mm Hg respectively was recorded as non- hypertensive while, Systolic and diastolic readings of 130mm Hg and above 80mm Hg respectively was recorded as hypertensive.

2.8. Diabetes screening

Fasting blood sugar test was carried out early in the morning to ensure the patients had not eaten. A glucometer was used for the routine diabetes tests as described by Cheesbrough (22). The fingertip was sterilized using a sterile swab and allowed to air dry. A sterile lancet that came with the kit was used to prick the side of a fingertip. The edge of a test strip was made to touch the drop of blood that arose after the finger prick and the strip placed in the meter. The record of result was noted. 99 mg/dL or lower indicated a normal fasting blood sugar level, 100–125 mg/dL indicated prediabetes while 126 mg/dL or above indicated high blood sugar, the main sign of diabetes.

2.9. Bacterial Isolation

The swabs were streaked on Blood agar, Nutrient Agar and MacConkey agar plates under aseptic measures and incubated aerobically for 24 hours at 37^{o}C as described by NCCLS (20). The swabs were also streaked on blood agar plates under aseptic measures and incubated anaerobically in an anaerobic jar for 48 hours at 37^{o}C as described by NCCLS (20). Bacterial growth on blood agar, nutrient agar and MacConkey were observed after the incubation. Different bacterial colonies were isolated and sub-cultured according to their colony morphology.

2.10. Characterization and identification of the isolates

The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacteria colony were colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation as described by Fawole and Oso (21).

2.10.1. Gram-staining

This was carried out as described by Fawole and Oso (21). A drop of distilled water was placed on a clean grease free glass slide and a colony of the isolate was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame four times to heat-fix. The smear was flooded with crystal violet and allowed to stay for 60 seconds and rinsed with distilled water. Lugol's iodine was added and allowed to stand for 60 seconds and rinsed with water and then decolorized with acetone for 10 seconds and rinsed with distilled water. The smear was counter stained with safranin for 60 seconds and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using x100 objective lens.

2.10.2. Catalase test

The method described by Cheesbrough (22) was used. One drop of hydrogen peroxide solution was introduced on a clean glass slide, followed by the inoculation of a 24-hour old culture on the slide. The presence of gas bubbles indicated a positive test while the absence of gas bubbles indicated negative reaction.

2.10.3. Citrate utilization test

The method described by Cheesbrough (22) was adopted. Simmon’s citrate agar was prepared in accordance with manufacturer’s manual. 24.28 g of the media was weighed and dissolved in 1000ml of water. The medium was boiled for 15min, the dissolved medium was then dispensed into tubes and sterilized in an autoclave at 15 lbs. pressure 121°C for 15 minutes. The tubes were taken out and cooled at a slanted position to a temperature of 40°C. A well-isolated

colony was taken from a 24-hour culture with a sterile inoculating needle and inoculated by streaking the surface of the citrate agar slants. The test tubes caps were left loosened to ensure adequate aeration. The tubes were incubated aerobically at 37°C for 4 days. Change in color indicated a negative result.

2.10.4. Coagulase test

This was carried out as described by Olutola *et al*. (23). Five milliliters of blood were collected, transferred into EDTA container and centrifuged. A drop of physiological saline was added to each end of a clean glass slide in which one was used as control. A smear was made using a 24-hour old isolate of the test organism and physiological saline on the glass slide. A drop of human plasma was added into the test smear to make a suspension. Clumping within 10 seconds indicated a positive result which implied the ability of the test organisms to produce coagulase, an enzyme that coagulates blood plasma while for a negative result, no clumping was observed.

2.10.5. Urease test

The method described by Olutola*et al*. (23) was used. Urease agar was prepared in accordance with manufacturer's manual,24.52 g of the media was weighed and dissolved in 950ml of water. The prepared suspension was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes. The beaker was allowed to cool to 50°C. To the beaker, 50 ml of sterile 40% urea solution was added and mixed well. The medium was dispensed into tubes and set in a position to obtain agar slants. A loopful of a well-isolated colony was taken with a sterile inoculating loop and inoculated on the agar slants. The tubes were incubated with loosened caps at 37°C.The tubes were observed for the development of pink color for 7 days.

2.10.6. Motility test

The method described by Cheesbrough (22) was adopted. Sulphur Indole Motility (SIM) Medium was prepared in accordance with manufacturer's manual. 30 g of the media was weighed and dissolved in 1000ml of water, heated to boiling with agitation to completely dissolve. The prepared suspension was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes. The beaker was allowed to cool to 50°C. The medium was dispensed into tubes and allowed to gel. A straight inoculating loop was used to inoculate the tubes with a colony of 24-hour culture by stabbing once to a depth of only 1/3 to ½ inch in the middle of the tube. The inoculating loop was kept in the same line it entered as it is removed from the medium. The tubes were incubated at 37°C for 24 hours and examined daily for a diffuse zone of growth flaring out from the line of inoculation.

2.10.7. Methyl red test

The methyl red (MR) test detects the production of sufficient acid such as lactic acid, acetic acid or formic acid during the fermentation of glucose. The medium (glucose phosphate peptone water) was allowed to equilibrate to room temperature before lightly inoculating with organisms taken from a 24-hour culture. It was aerobically Incubated at 37°C for 24 hours. After 24 hours of incubation, 1ml of the broth was introduced to a clean test tube. The remaining broth was reincubated for an additional 24 hours. Two drops of methyl red indicator were added to the aliquot and observed for red color immediately as described by NCCLS (20).

2.10.8. Hydrogen sulfide test

The method described by Cheesbrough (22) was used. Sulphur Indole Motility (SIM) medium was prepared in accordance with manufacturer's manual. 30 g of the media was weighed and dissolved in 1000ml of water, heated to boiling with agitation to completely dissolve. The prepared suspension was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes. The beaker was allowed to cool to 50°C. The medium was dispensed into labelled tubes and allowed to gel. The organisms were Inoculated into the labeled tubes by means of stab inoculation and Incubated at 37°C for 24 hours. Formation of black precipitate indicated a positive reaction.

2.10.9. Voges–proskauer test

The method described by Cheesbrough (22) was adopted. 17 g of the glucose phosphate peptone water was weighed and dissolved in 1000ml of water, heated to boiling with agitation to completely dissolve. Five ml of the broth was dispensed in a clean test tube and loosely cotton plugged. The prepared suspension was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes. The medium was allowed to equilibrate to room temperature before lightly inoculating with organisms taken from a 24-hour culture. It was aerobically incubated at 37°C for 24 hours. After 24 hours of incubation, 2ml of the broth was transferred to a clean test tube. The remaining broth was re-incubated for an additional 24 hours. Six drops of 5% alpha-naphthol were added and mixed well to aerate. Two drops of 40% potassium

hydroxide were added and mixed well to aerate. Pink-red color at the surface within 30 minutes indicated a positive reaction.

2.10.10. Spore test

A smear of 24-hour culture was prepared, air-dried and heat-fixed. A beaker of water was placed on the hot plate and boiled until steam started coming up from the water. The hot plate was then turn down so that the water was barely boiling. The wire stain rack was placed over the beaker which had steam coming up from the boiled water. A small piece of paper towel cut and placed on top of the smear on the slide. The smear was flooded with the primary dye, malachite green, and left for 5 minutes. The paper towel was kept moist with the malachite green ensuring that the dye did not dry on the towel. The small paper towel was removed and discarded. The smear was flooded with the counterstain dye, safrinin, and left for 1 minute. It was washed well with water and blotted dry with filter paper. Light green colouration indicated spore formation while pink colour indicated non spore formation as described by NCCLS (20).

2.10.11. Sugar fermentation test

This was carried out as described by Olutola *et al*. (23). Purple Broth consisting of peptone and bromcresol purple was prepared in accordance with manufacturer's manual by weighing and dissolving 15.02 g of the media in 1000ml of water, heated to boiling with agitation to completely dissolve. The prepared suspension was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 minutes. The Purple Broth was allowed to warm to room temperature before inoculation (with glucose, maltose, sucrose, lactose, fructose) with isolated colonies from a 24-hour culture of the organism. A control tube of Purple Broth Base was inoculated in parallel with the carbohydrate-based media. Inoculated media was aerobically incubated at 37ºC. for 48 hours. Development of a yellow color in the medium was an indication of positive result.

2.10.12. Indole test

The method described by Cheesbrough (22) was adopted 16g of Tryptophan Broth was dissolved in one liter of water by heating. 3 ml was dispensed per test tube and closed the with cotton plugs. The test tubes were autoclaved for 15 min at 121 °C. A sterilized test tubes containing 4 ml of tryptophan broth was aseptically inoculated with isolated colony from a 24-hour culture of the organism, and incubated the tube at 37°C for 24 hours. 0.5 ml of Kovac's reagent was added to the broth culture and observed for the presence or absence of ring. Formation of a pink to red colour in the reagent layer on top of the medium within seconds of adding the reagent records a positive test while, no colour change indicated a negative test result.

2.10.13. Oxidase test

This was carried out as described by Cheesbrough (22) 1.0 grams of N, N, N, N-tetramethyl-p-phenylenediamine dihydrochloride (oxidase reagent) was dissolved in 100 mL of sterile distilled water and mixed well. 2 drops of the reagent were directly added to isolated colony from a 24-hour culture of the organism and observed for color change within 10 seconds. Colour change to dark purple within 5 to 10 seconds was a positive test while, no colour change longer than 2 minutes indicated a negative test result.

2.11. Antibiotics susceptibility testing

Antibiotics susceptibility testing (AST) was carried out for the isolated bacteria with Penicillin, Oxacillin, Cefoxitin, Gentamycin, Amikacin, Doxycycline, Ciprofloxacin, Bacitracin, TTrime, Chloramphenicol, Erythromycin, Clindamycin, Vancomycin, Amikacin, Tobramycin, Doxycycline, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Cefepime, Piperacillin-Tazobactam, Ampicillin-Sulbactam, Augmentin, Ceftriaxone, Cefotaxime, Ceftazidime, Imipenem Meropenem and Aztreonam antibiotics on Mueller Hinton Agar (MHA) using the Kirby Bauer disk diffusion technique according to CLSI (24) guidelines. The inoculum for each isolate was prepared by emulsifying colonies from the purified culture overnight in normally sterile saline in test tubes with turbidity adjusted to standard 0.5 McFarland. The bacterial suspension was spread evenly on the MHA plate with a sterile swab, left for 3 minutes, and then the antibiotic discs were applied. The plates were incubated at 35°C for 24 hours, and the diameters of the zone of inhibition were measured with a Vernier caliper, and the results were interpreted as resistant, intermediate or sensitive.

2.12. Statistical Analysis of Results

Statistical analysis was carried out to determine the significant difference in the antibiotics sensitivity patterns of the isolated bacteria to the tested antibiotics using Statistical Package for Social Sciences (SPSS).

3. Results

The distribution of the study participants is presented in Table 1. It showed that 117 study participants from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital 68(58.1%), St Charles Medical and Diagnostics 21(18.0%) and Graceland Specialist Hospital and Maternity 28(23.9%) were included in the present study.

Table 1 Distribution of study participants

Study sites	Study participants	Frequency (%)
COOUTH	68	58.1
SCMD	21	18.0
GSHM	28	23.9
Total	117	100

Key: COOUTH= Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, SCMD= St. Charles Medicals and Diagnostics, GSHM= Graceland Specialist Hospital and Maternity.

Table 2 showed the distribution of study participants according to sex; out of the 117 study participants, 84(71.8%) were male and 33(28.2%) female as shown in table 2.

Table 2 Distribution of study participants according to Sex

Sex	Study participants	Frequency (%)
Male	84	71.8
Female	33	28.2
Total	117	100

The distribution of study participants according to age is shown in Table 3. The result showed that out of the 117 study participants, most participants were between 51 and 60 years old.

Table 3 Distribution of study participants according to age

Age	Study participants	Frequency (%)
<40	5	4.3
41-50	32	27.4
51-60	45	38.4
61-70	22	18.8
71-80	12	10.3
>81	1	0.8
Total	117	100

Table 4 showed the distribution according to socio-economic status of study participants with motor vehicle drivers having the highest occurrence (22.1%).

Table 4 Distribution of participants according to socio-economic status (occupation)

Occupation	Study participants	Frequency (%)
Farmers	19	16.1
Housewives	5	4.2
Traders	15	12.9
Office workers	11	9.3
Motor vehicle drivers	26	22.1
Building construction workers	14	11.9
Auto mechanics	14	11.9
Students	2	1.6
Metal construction workers	12	10.2
Total	117	100

The wound site distribution among the study participamts is shown in Table 5. The wound samples were collected from participants different sites as recorded, with the highest number taken from heels 57(48.7%)

Table 5 Wound site distribution among study participants

Wound site	Study participants	Frequency (%)
Hammer toe tip	53	45.3
Hand	7	6.0
Heel	57	48.7
Total	117	100

Table 6 showed the study participants wound grading according to Wagners classification with grade 3 having the highest frequency 56(47.9%), followed by grade 2 and 4.

Table 6 Wound Grading according to Wagners' classification among study participants

Wound grade	Study participants	Frequency (%)
Grade 1	2	1.7
Grade 2	37	31.6
Grade 3	56	47.9
Grade 4	21	17.9
Grade 5	1	0.9
Total	117	100

The distribution of diabetes types among study participants is presented in Table 7. This study recorded three types of diabetes amount participants; Type-1, Type-II and Type-III. The majority of the participants had type I diabetes.

Table 7 Distribution of diabetes types among study participants

Type of diabetes	Study participants	Frequency (%)
Type I	58	49.0
Type II	54	46.0
Gestational	7	5.0
Total	117	100

Table 8 showed the distribution of diabetes duration among the study participants. Most of the participants had lived with diabetes for periods of 1-10 years.

Table 8 Distribution of diabetes duration among study participants

Year	Study participants	Frequency (%)
<1	0	0
1-10	65	55.6
11-20	33	28.2
21-30	18	15.3
>31	1	0.9
Total	117	100

The other clinical characteristics of study participants are shown in Table 9. The result showed the clinical characteristics of study participants such as HGBAIC levels in which majority was within 9.67-16.1mmol/mol levels. Other diabetes related disease data like hypertension, kidney disease, PN and PVD are shown

Table 9 Other clinical characteristics of study participants

Characteristics	Category	Study participants	Frequency (%)
HGBAIC	1.6-8.06mmol/mo 9.67-16.11mmol/mol 17.72-24.17mmol/mol ≥24.17mmol/mol	0 69 47 1	0 58.9 40.2 0.9
Hypertension	Yes No	63 54	53.8 46.2
Kidney disease	Yes No	15 102	12.8 87.2
Skin texture	Dry Moist Cracked	82 9 26	70.1 7.7 22.2
PVD	Yes No	100 17	85.5 14.5
PN	Yes No	99 18	84.6 15.4

Key: HGBAIC= Hemoglobin A1C, PVD= Peripheral Vascular Disease, PN= Peripheral Neuropathy.

Table 10 showed the cultural characteristics of the cultured microorganisms on nutrient agar. The shape, size, elevation, surface, colour and structure/appearance of the colonies were used as parameters to indicate isolates suspected in the medium.

Table 10 Cultural characteristics of the isolates on nutrient agar

Shape	Size(mm)	Elevation	Surface	Colour	Structure/ Appearance	Suspected microorganism
irregular	2.5	low convex	smooth	greenish	translucent	*Pseudomonas aeruginosa*
circular	2.5	convex	smooth	golden-yellow	opaque	*Staphylococcus aureus*
circular	2	convex	smooth	greyish-white	translucent	*Escherichia coli*
circular	2.2	dome- shaped	mucoid	greyish-white	translucent	*Klebsiella pneumoniae*
irregular	1.3	effuse	glistening	greyish-white	translucent	*Proteus vulgaris*
circular	2	convex	mucoid	greyish-white	opaque	*Enterobacter aerogenes*
circular	3	raised	smooth	red	opaque-whitish	*Serratia marcescens*
spherical	1.3		smooth	white	opaque	*Enterococcus faecalis*
circular	0.7	low convex	matt	light yellow	opaque	*Streptococcus pyogenes*
circular	1	raised		creame-	transparent	*Staphylococcus epidermidis*

Table 11 showed the cultural characteristics of the cultured microorganisms on MacConkey agar. The shape, size, elevation, surface, colour and structure/appearance of the colonies were used as parameters to indicate isolates suspected in the medium.

Table 11 Cultural characteristics of the isolates on MacConkey agar

Shape	Size(mm)	Elevation	Surface	Colour	Structure/ Appearance	Suspected microorganism
circular	2.1	low convex	smooth	colourless	transparent	*Pseudomonas aeruginosa*
circular	2.5	convex	smooth	pink	opaque	*Staphylococcus aureus*
circular	2	convex	smooth	pink	opaque	*Escherichia coli*
circular	2.1	convex	mucoid	red	opaque	*Klebsiella pneumoniae*
circular	2.2	low convex	smooth	colourless	transparent	*Proteus vulgaris*
circular	2.1	convex	mucoid	pink	opaque	*Enterobacter aerogenes*
circular	3	raised	smooth	pink	transparent	*Serratia marcescens*
spherical			smooth	pink		*Enterococcus faecalis*
circular	0.6	low convex	matt	light golden- yellow	Semi-transparent	*Streptococcus pyogenes*

Table 12 showed the cultural characteristics of the cultured microorganisms on blood agar. The shape, size, elevation, surface, colour, hemolysis and structure/appearance of the colonies was used as parameters to indicate isolates suspected in the medium.

Table 12 Cultural characteristics of the isolates on Blood agar

Shape	Size(mm)	Elevation	Surface	Colour	Structure/ Appearance	Hemolysis	Suspected microorganism
irregular	2.5	flat	smooth	greyish white	translucent	β-Hemolysis	*Pseudomonas aeruginosa*
convex	2.7	convex	smooth	golden-yellow	opaque	β-Hemolysis	*Staphylococcus aureus*
circular	1.1	convex	smooth	greyish-white	translucent	β-Hemolysis	*Escherichia coli*
circular	2.2	dome-shaped	mucoid	greyish-white	opaque	γ-Hemolysis	*Klebsiella pneumoniae*
irregular	1.3	effuse	glistening	greyish-white	opaque	γ-Hemolysis	*Proteus vulgaris*
circular	2.6	entire	smooth	greyish-white	shiny		*Bacteroide fragilis*
circular	2	convex	mucoid	greyish-white	opaque		*Enterobacter aerogenes*
circular	2.1	raised	smooth	red		a-Hemolysis	*Serratia marcescens*
circular		convex	smooth	gray		non-hemolytic	*Enterococcus faecalis*
circular	0.9	low convex	matt	yellow	opaque	β-Hemolysis	*Streptococcus pyogenes*

Table 13 showed the Gram reaction of the different bacteria that were isolated and identified. A total of one hundred and fifteen bacterial isolates were isolated from 117 wound samples. Out of the 115 bacterial isolates, 37(32.2%) were Gram-positive, and 78(67.8%) were Gram negative isolates.

Table 13 Gram reaction of the isolates

Suspected bacterial isolates	Gram stain reaction
Serratia marcescens	negative-rod
Pseudomonas aeruginosa	negative-rod
Staphylococcus aureus	positive-cocci
Enterococcus faecalis	positive-cocci
Streptococcus pyogenes	positive-cocci
Escherichia coli	negative-rod
Klebsiella pneumoniae	negative-rod
Proteus vulgaris	negative-rod
Staphylococcus epidermidis	positive-cocci

Bacteroide fragilis	negative-rod
Enterobacter aerogenes	negative-rod

Table 14 showed the biochemical test results of the isolates. Different reagents as listed above were reacted with the isolates and recorded respectively.

Table 14 Biochemical reactions of the isolates

Isolates	Ind	Ure	Mot	Cit	Cat	Met	Oxi	Coa	Hs	VP	SP	Bacterial isolates
1	-	-	+	+	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
2	-	+	-	+	+	+	-	+	-	+	-	*Staphylococcus aureus*
3	+	-	-	-	-	+	-	-	-	-	-	*Escherichia coli*
4	-	+	+	+	+	+	-	-	-	+	-	*Klebsiella pneumoniae*
5	+	+	+	-	+	+	-	-	+	-	-	*Proteus vulgaris*
6	-	+	+	+	+	-	-	-	-		-	*Bacteroide fragilis*
7	-	-	+	+	+	-	-	-	-	+	-	*Enterobacter aerogenes*
8	-	-	+	+	+	-	-	-	-	+	-	*Serratia marcescens*
9	-	-	-	-	-	-	-	-	-	+	-	*Enterococcus faecalis*
10	-	-	-		-	+	-	-		-	-	*Streptococcus pyogenes*
11	-	+	-	-	+	-	-	-	+	-	-	*Staphylococcus epidermidis*

Keys: (-) =Negative (+) =Positive Mot=Motility Cit=Citrate Met=Methyl Red Oxi=Oxidase Cat=Catalase Coa=Coagulase Ind = Indole Ure = Urease Hs =Hydrogen Sulfide VP= Voges–Proskauer SP= Spore test

Table 15 showed the sugar fermentation result of isolates. Maltose, glucose, sucrose, lactose and fructose were the sugars used

Table 15 Sugar fermentation results of the isolates

Maltose	Glucose	Sucrose	Lactose	Fructose	Bacterial isolates
-	-	-	-	-	*Pseudomonas aeruginosa*
+	+	+	+	+	*Staphylococcus aureus*
-	+	+	+	-	*Escherichia coli*
+	+	+	+	+	*Klebsiella pneumoniae*
-	+	-	-		*Proteus vulgaris*
+	+	+	+		*Bacteroide fragilis*
+	+	+	-	+	*Enterobacter aerogenes*
+	+	+	-	+	*Serratia marcescens*
+	+	+	+	+	*Enterococcus faecalis*
+	+	+	+	+	*Streptococcus pyogenes*
+	+	+	+	+	*Staphylococcus epidermidis*

Keys: (-) =Negative (+) =Positive

The Frequency of isolation of the bacterial isolates is presented in Table16. One hundred and fifteen bacterial isolates were identified from 117 patients with diabetic wounds. *Staphylococcus aureus* had the highest frequency (25.2%) while *Streptococcus pyogenes* had the lowest frequency of isolation of 0.9%.

Table 16 Frequency of isolation of the bacterial isolates

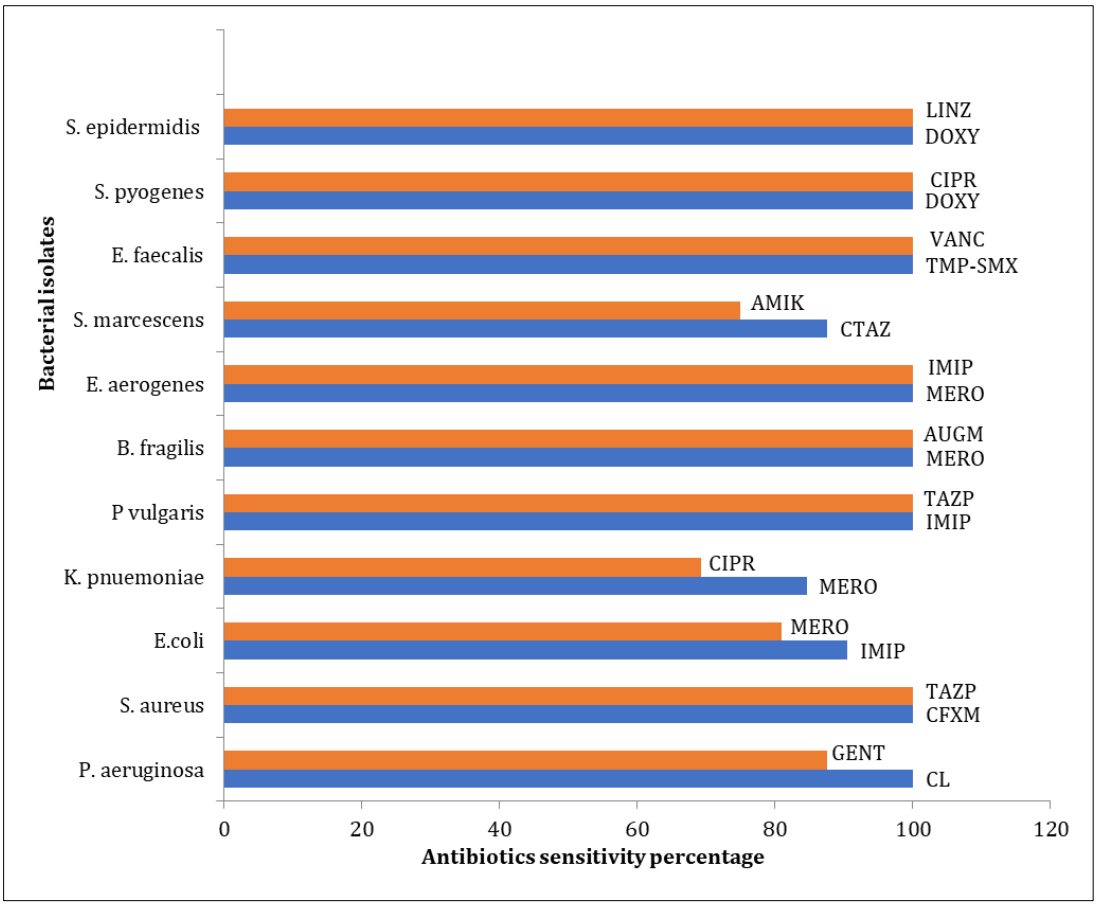
Isolated bacteria	Number isolated	Frequency of isolation (%)
Pseudomonas aeruginosa	24	20.8
Staphylococcus aureus	29	25.2
Escherichia coli	21	18.3
Klebsiella pneumoniae	13	11.3
Proteus vulgaris	5	4.3
Bacteroide fragilis	3	2.6
Serratia marcescen	8	7.0
Enterococcus faecalis	4	3.5
Streptococcus pyogenes	1	0.9
Enterobacter aerogenes	4	3.5
Staphylococcus epidermidis	3	2.6
Total	115	100

The frequency of pure and mixed culture plates is shown in Table 17. Pure cultures were 34(29.6%), while mixed culture were plates 81(70.4%).

Table 17 Frequency of pure and mixed culture plates

Isolated bacteria	Study participants	Frequency (%)
Pure culture	34	29.6
Mixed culture	81	70.4
Total	115	100

The most effective antibiotics against the isolated bacteria are shown in Figure 4.



Key: GENT=Gentamicin, DOXY=Doxycycline, VANC=Vancomycin, LINZ=Linezolid, CTAZ=Ceftazidime, TAZP=Piperacillin-tazobactam, IMIP=Imipenem, MERO=Meropenem, CL=Colistin, AMIK=Amikacin, CFXM=Cefuroxime, TMP-SMX=Trimethoprim-sulfamethoxazole, CIPR=Ciprofloxacin, AUGM=Augmentin.

Figure 4 Most effective antibiotics against the isolated bacteria.

The antibiotics resistance patterns of the bacterial isolates are presented in Table 18. Multidrug-resistance (MDR) profiles of the organisms showed that of the 115 bacterial isolates, 99 (86.1%) were MDR, that is resistant to more than two agents of antibiotic classes, whereas 16(13.9%) were non-MDR.

Table 18 Antibiotics resistance patterns of the bacterial isolates.

Isolates	Antibiogram pattern and Frequency (%)											
	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	MDR
Pseudomonas aeruginosa	0	0	2(8.3)	4(16.7)	3(12.5)	5(20.8)	5(20.8)	1(4.2)	4(16.7)	0	0	22(91.7)
Staphylococcus aureus	0	0	3(10.3)	3(10.3)	7(24.1)	5(17.2)	5(17.2)	2(6.9)	4(13.8)	0	0	26(89.7)
Escherichia coli	0	0	2(9.5)	4(19.0)	6(28.6)	3(14.3)	4(19.0)	1(4.8)	1(4.8)	0	0	19(90.5)
Klebsiella pneumoniae	0	0	1(7.7)	1(7.7)	(15.4)	4(30.8)	2(15.4)	0	2(15.4)	1(7.7)	0	12(92.3)
Proteus vulgaris	0	1(20.0)	0	0	3(60.0)	1(20.0)	1(20.0)	0	0	0	0	4(80.0)
Bacteroide fragilis	0	0	1(33.3)	0	0	1(33.3)	0	0	0	1(33.3)	0	2(66.7)

Serratia marcescens	0	0	2(25.0)	0	0	0	5(62.5)	1(12.5)	0	0	0	6(75.0)
Enterococcus faecalis	0	1(25.0)	0	0	0	0	1(25.0)	0	2(50.0)	0	0	3(75.0)
Streptococcus pyogenes	0	0	0	0	0	0	0	0	1(100)	0	0	1(100)
Enterobacter aerogenes	0	1(25.0)	1(25.0)	0	(50.0)	0	0	0	0	0	0	2(50.0)
Staphylococcus epidermidis	0	0	1(33.3)	0	0	2(66.7)	0	0	0	0	0	2(66.7)

Table 19 showed the incidence of antibiotics that the isolated bacteria were most resistant to. Isolate incidence as shown, represents the total number of a particular bacteria isolated while, most ineffective incidence records the most number that was not sensitive to the antibiotics respectively.

Table 19 Most ineffective antibiotics against the isolated bacteria

Isolated bacteria	**Number of isolates**	**Antibiotics**	**Most ineffective frequency (%)**	**Percentage frequency (%)**
Pseudomonas aeruginosa	24	Vancomycin Erythromycin	24 24	100 100
Staphylococcus aureus	29	Meropenem Ceftazidime	29 27	100 100
Escherichia coli	21	Cefoxitin Erythromycin	21 21	100 100
Klebsiella pneumoniae	13	Vancomycin Doxycycline	13 13	100 100
Proteus vulgaris	5	Chloramphenicol Polymyxin	5 5	100 100
Bacteroide fragilis	3	Tobramycin Cefoperazone	3 3	100 100
Serratia marcescens	8	Ceftriaxone Augmentin	8 8	100 100
Enterococcus faecalis	4	Erythromycin Clindamycin	4 4	100 100
Streptococcus pyogenes	1	Augmentin Ampicillin	1 1	100 100
Enterobacter earogenes	4	Polymyxin Cefoxitin	4 4	100 100
Staphylococcus epidermidis	3	Ampicillin Erythromycin	3 3	100 100

4. Discussion

One of the serious consequences of diabetes is diabetic wound infections like, diabetic foot infections and infected diabetic foot ulcer. Diabetic foot ulcers, if left untreated, can become infected and cause other complications, such as gangrene, osteomyelitis (local spread of infection to muscle and bone), and amputation. Surgery and antibiotic therapy are the options used to control this infection. From time to time, the pattern of bacterial profile and their antibiotic susceptibility pattern changes from one region to another within the country and also between the countries. Lack of updated knowledge among physicians regarding the microbial profile and their antibiotic susceptibility pattern in a locality will hinder the selection of appropriate empirical antibiotic therapy of diabetic wound infection for the best outcome (25).

Table 1 showed that one hundred and seventeen study participants from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital 68(58.1%), St Charles Medical and Diagnostics 21(18.0%) and Graceland Specialist Hospital and Maternity 28(23.9%) were included in the present study which is in line with the study conducted in Ethiopia (19). The study sites are situated at the center of the town and often have a high influx of patients which broadens the scope of achieving a robust random sampling.

A male predominance in the study participants was noted in this study (Table 2), in line with previous studies in Indonesia and India (26, 27). This may be explained by the more active role of men in outdoor activities such as motor vehicle driving and farming, leading to injuries and exposure to the development of wounds. Similarly, the majority of participants with diabetic wound infections were found to be within the age range of 51-60 years (Table 3), in agreement with the same studies in India and Indonesia (26, 28). Increased prevalence among the elderly may be due to multiple reasons such as longer duration of diabetes mellitus, the presence of multiple co-morbidities and reduced immune status (12). The male predominance in this study was evident in Table 4 that showed the distribution of participants according to their socio-economic status, where motor vehicle drivers (22.1%) had the highest frequency followed by farmers (16.1%).

The wound samples were collected from different sites from the participants, with the highest number taken from heel 57(48.7%) (Table 5). This is in line with previous studies by Ogba *et al.,* (30). This can be explained with the fact that patients who have diabetes for many years can develop neuropathy, a reduced or complete lack of ability to feel pain in the feet due to nerve damage as such pays little or no attention to sustained wounds until it exacerbates.

In this study, wounds were classified according to the Wagner Diabetic Foot Ulcer Classification System (Table 6). The most common was grade 3 at 56(47.9%) followed by grade 2 at 37(31.6%), which is consistent with the research conducted in Ethiopia, where grade 3 was found in 50.3% (64/127) followed by grade 2 in 26.7% (34/127) of participants (19). Contrary to these findings, a study from India showed that grade 2 (69.2%) was higher than grade 3 (5.1%) (31). The Wagners classification was; Grade 0-Pre-ulcerative, with no open lesion or cellulitis, Grade1-Superficial ulcer, Grade2-Deep ulcer up to tendons and joint tissue, Grade 3-Deep ulcer with abscess, osteomyelitis, and joint sepsis, Grade 4- Localized gangrene of forefoot or heel, and Grade 5-Gangrene of entire foot/global gangrene.

The types and duration of diabetes among study participants were also studied. Three diabetes types were shown; Type-1, Type-II and Type-III with majority of the participants having type I diabetes (Table 7). Also, most of the participants have lived with diabetes for periods of 10-20 years (Table 8) which is in line with a study conducted by Asegdew *et al.* (19) on bacterial isolates from diabetic foot ulcers and their antimicrobial resistance profile from selected hospitals in Addis Ababa, Ethiopia. Other clinical characteristics of study participants which comprised of the co-morbidities that increases the prevalence of diabetic wound infections among the elderly was evident in Table 9. Among the co-morbidities, Peripheral Vascular Disease was the most commonly associated co-morbidity, followed by Peripheral neuropathy and hypertension. Peripheral Vascular Disease increases the risk of microvascular and macrovascular complications. Common devastating problems of the diabetic wound infection are osteomyelitis and lower limb amputation (29).

The shape, size, elevation, surface, colour and appearance/structure of the colonies present on different media were used as parameters for cultural characteristics result of cultured microorganisms (Tables 10, 11 and 12). A total of one hundred and fifteen bacterial isolates were isolated from 117 wound samples (Table 13). Out of the 115 bacterial isolates, 37(32.2%) were Gram-positive, and 78(67.8%) were Gram negative isolates. The suspected isolates reaction tests to Citrate, Methyl Red, Oxidase, Catalase, Coagulase, Indole, Urease, Hydrogen Sulfide, Voges–Proskauer, Spore formation, Motility (Table 14) and sugar fermentation test results as recorded in Table 15 were further used in identification of bacterial isolates.

In this study, a high growth rate 115(98.3%) of the bacteria was found (Table 16) comparable with a study in Ethiopia with a growth rate of 77.3% (92/119), and also compared to no growth at 22.7% (27/119) (45). A recent study also reported that the growth rate was 81.7% (98/120), and no growth of 18.3% (22/120), respectively (3I). *Staphylococcus aureus* was the predominant isolate 29(25.2%) (Table 16). This finding is in agreement with an earlier study done in Ethiopia and Oman where *Staphylococcus aureus* 25.19% (32/127) and 44(18.9%) respectively were predominant, unlike a previous study in Ethiopia which reported, *Klebsiella species* 23.9% (22/92) as the predominant bacteria followed by *Proteus species* 18.47% (17/92) (6, 27, 5). In Egypt, *P. mirabilis* (16.8%) was the most common isolate (32), in Saudi Arabia Pseudomonas species 134(15.6%) (33), and in South America *Pseudomonas species* (18.8%) was the most common isolate (34). Similarly, in agreement with studies in Kenya 17.5% (14/80) (35), Nigeria 32.9% (32/97) (30), India 24.42% (32/131) (36), China 232(65.2%) (37), and in Iran 92(28%) (38). This showed that the predominant bacteria causing diabetic wound infections could vary in different settings.

Overall, Gram-negative bacteria 78(67.8%) were predominantly isolated compared to Gram-positive isolates 37(32.2%) (Table 16). This finding is in agreement with an earlier study done in Oman, where gram-negative bacteria were isolated in 175(75%) versus 58(25%) gram-positive bacteria (5). Similarly, a study from Egypt reported 56% Gram-negative and 27.7% Gram positives, while 79% Gram-positive and 21% Gram negatives were isolated in northeast India (31, 32).

The rate of bacterial isolation and the type of bacteria in the wounds increased as the severity of the wound increased. This shows the extent to which organisms influence the diabetic wound infection healing process, which is supported by various papers published elsewhere, such as in Nigeria (39), China (37), and India (27, 40).

In the present study, microbiological evaluation revealed that diabetic wound infections are often polymicrobial 81(70.4%) against pure cultures 34(29.6%) as shown in Table 17, this could be because the wound samples that produced mixed cultures had been cross-contaminated by other etiologic agents due to poor wound care or wound grade according to Wagners' diabetic wound classification are at their late levels. Overall, infection was caused by pure isolates 34(29.6%), and mixed culture 81(70.4%) samples. In general, as the degree of wound increases, the percentage with polymicrobial growth increases (Table 17). This result is similar to previous studies (41, 17, 24). Regarding the predominance of the etiological agent in diabetic wound infections, several studies have shown diversity in the pathogens associated with the infections (41, 18, 42, 43). The current study found that the most common Gram-negative isolates were *Pseudomonas aerugnosa* 24(20.8%), followed by *Escherichia coli* 21(18.3) (Table 16), comparable with other studies conducted in Libya 17.5% (21/120) (44), India 85(23.2%) (40). On the other hand, another study in Ethiopia reported that no *Pseudomonas species* were isolated from 92 cultured samples, whereas *Escherichia coli* was isolated in 5.43% (5/92) (45). Similarly, in Pakistan, the most common Gram-negative bacteria was *Escherichia coli* 15.72% 671(15.72%) (46). This variation may be due to the sample size differences of the different studies and other unique characteristics of each study site.

A very high rate of multidrug resistance 99(86.1%) was found in the present study (Table 18). Multidrug-resistance (MDR) profiles of the organisms showed that of the 115 bacterial isolates, 99 (86.1%) were MDR, that is resistant to more than two agents of antibiotic classes, whereas 16(13.9%) were non-MDR. 26(89.7%) of *Staphylococcus aureus* isolates were MDR. *Pseudomonas aerugnosa*, *Klebsiella pneumoniae* and *Escherichia coli* isolates were resistant to all types of antibiotics. However, the MDR profiles within species vary as reported in the findings of a study in Nigeria (47) and India (47). Gram-negative organisms are known to develop resistance to multiple antibiotics rapidly, compared to Gram-positive etiologic agents. In the last two decades, there is a rapid increase in the rate of infections caused by multidrug resistance Gram-negative pathogens as demonstrated by many studies (48, 13, 49).

Colistin was most effective against *Pseudomonas aeruginosa*(100%) followed by Gentamicin (87.5%), Cefuroxime was most effective against *Staphylococcus aureus* (100%) followed by Piperacillin-tazobactam (100%), Imipenem was most effective against *Escherichia coli* (90.5%) followed by Meropenem (81.0%), Meropenem was most effective against *Klebsiella pneumoniae* to (84.6%) followed by Ciprofloxacin (69.2%), Imipenem was most effective against *Proteus vulgaris* (100%) followed by Piperacillin-tazobactam (100%), Meropenem was most effective against *Bacteroide fragilis* (100%) followed by Augmentin (100%), Meropenem was most effective against *Enterobacter aerogenes* (100%) followed by Imipenem (100%), Ceftazidime was most effective against *Serratia marcescens* (87.5%) followed by Amikacin (75.0%), Trimethoprim-sulfamethoxazole was most effective against *Enterococcus faecalis* (100%) followed by Vancomycin (100%), Doxycycline was most effective against *Streptococcus pyogenes* (100%) followed by Ciprofloxacin (100%), Doxycycline was most effective against *S. epidermidis* (100%) followed by Linezolid (100%) (Figure 4).

*Pseudomonas aeruginosa*was most resistant to Vancomycin (100%) followed by Erythromycin (87.5%), *Staphylococcus aureus* was most resistant to Meropenem (100%) followed by Ceftazidime (100%), *Escherichia coli* was most resistant to Cefoxitin (100%) followed by Erythromycin (100%), *Klebsiella pneumonia* was most resistant to Vancomycin (100%) followed by Doxycycline (100%), *Proteus vulgaris* was most resistant to Chloramphenicol (100%) followed by Polymyxin (100%), *Bacteroide fragilis* was most resistant to Tobramycin (100%) followed by Cefoperazone (100%), *Enterobacter* sp. was most resistant to Meropenem (100%) followed by Imipenem (100%), *Serratia marcescens* was most resistant to Ceftriaxone (100%) followed by Augmentin (100%), *Enterococcus faecalis* was most resistant to Erythromycin (100%) followed by Clindamycin (100%), *Streptococcus pyogenes* was most resistant to Augmentin (100%) followed by Ampicillin (100%), *S. epidermidis* was most resistant to Ampicillin (100%) followed by Erythromycin (100 (Table 19).

5. Conclusion

Diabetic wounds can be infected with a wide variety of pathogens and a large number of multi-drug resistant bacteria. In this study, *Staphylococcus aureus* was the dominant isolate, followed by *Pseudomonas aeruginosa*and other Gram-negative bacteria. A high level of resistance to commonly used antibiotics such as Vancomycin, Erythromycin, Ceftazidime, Chloramphenicol was also observed, highlighting the need for caution in the use of antibiotics for the treatment of infections. A high number of diabetic wound infections with multi-drug resistance was also recorded among patients with high predisposing risk factors like age and socio-economic status. Close attention given to this class of patients would help reduce the development and spread of antibiotic resistant strains. Antimicrobial susceptibility testing should be carried out on isolates of diabetic wound infections before chemotherapy to avoid selection of drug resistant strains. This study recommends the use of carbapenems like Meropenem and Doxycycline for empirical therapy of Gram-negative and Gram-positive bacteria of diabetic wound infections, respectively. In the course of this study conducted within Awka, Anambra state, it was observed that Piperacillin-tazobactam antibiotic was efficacious in inhibiting the growth of Gram positive and negative bacteria as well as aerobic and anaerobic bacteria, Thus, it can be an empirical choice for treatment of diabetic wound infections.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical clearance was sought from the Ethics and Research Committee of the three hospitals. Permission to collect samples from consented patients was obtained from the Hospital Head of Laboratory Medicine. Semi-structured questionnaires were used to collect sociodemographic and other clinical data such as; sex, age, type of diabetes, duration of diabetes, HGBA1C, hypertension, kidney disease and leg skin texture after an informed consent was obtained from each participants of the study. For those who were younger than 18, their parents or guardians were asked for their consent. Confidentiality was maintained by omitting their personal identifiers throughout the study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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